STUDIES OF DIPYRONE (METAMIZOLE SODIUM) TOXICITY IN AVIAN SPECIES

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ABSTRACT

The anti-pyretic and analgesic effects of dipyrone are similar to those of the other drugs but its toxicity differs significantly. Despite of sharing clinical usefulness, dipyrone share several unwanted side effects, the most common is a propensity to induce sudden and unidentified emergency that can sometimes result into death of patient (Gabriel *et al*., 1991). Which restrict its prescription in human, poultry and veterinary practice. Thus it is needed to study dipyrone to prevent causalities, find out toxicity and reduce angiogenic factor in clinical practice. We therefore aimed to determine toxicity of dipyrone to assure the safety of wild life. Different toxic levels of dipyrone were administered intra-muscularly to broiler chicks for four days. Feed and water were provided *ad libitum*. A record of physical examination, signs of toxicity and mortality rate in each group was maintained. Blood samples were drawn before and after medication for examination of AST, ALT, Uric Acid, Alkaline phosphatase and Creatinine. Finally, postmortem examination was performed to confirm tissues damages. Based on the necropsy findings and biochemical analysis, it could be suggested to dipyrone in the avian species. Keeping in view the environmental problem; vultures crises, veterinary causalities, it is conceivable that dipyrone exerts desirable pharmacological effects in human medicine and may be used instead of diclofenac sodium in veterinary and poultry practices.

Keywords: Dipyrone (metamizole sodium) toxicity, Broiler birds, LFT's

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Introduction

Methane sulfonic acid sodium mono hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O$), Dipyrone or metamizole sodium is used as an analgesic and antipyretic agent. It has also been widely used by equine practitioners to treat equine colic and other conditions of gastrointestinal spasm or hypermotility in both small and large animals (Gray and Yano, 1975). Today the efficacy of dipyrone in certain spasmodic conditions in small and large animals is controversial (Lowe 1969; Gray and Yano 1975). According to Lowe (1969), dipyrone is valueless as an analgesic in the treatment of equine colic. It also has no effect on spontaneous intestinal motility or upon intestinal spasms produced by nerve stimulation, cholinergic drugs, ganglionic stimulant drugs, histamine, or morphine (Gray and Yano 1975). Conversely, the drug does antagonize bradykinin-induced intestinal spasms as do other members of the pyrazolon class of compounds. It is contraindicated in food-producing animals, including lactating dairy animals. Dipyrone can interfere with, or mask the presence of prohibited drugs in race animals for 5 days after its use (Bierhaus 1970). This would indicate that the drug should not be used in racing animals for at least 5 days prior to a race event. Moreover, recent information indicates that the use of dipyrone with chlorpromazine can result in serious hypothermia and it also has a tendency to increase bleeding, due to the suppression of the formation of prothrombin (Woodbury and Fingl 1975).Because of this contradictory discussion, we have aimed this project to evaluate the therapeutical credibility of metamizole sodium.

Therapeutical hazards of metamizole demand its re-evaluation to assure the safety of avian species being hazardous to wild life. Especially the already reported vulture crises and veterinary emergencies have increased the value of this project. Thus, it is direly need to find out a suitable replacement of diclofenac, prevent renal toxicity, induce tumor apoptosis and reduce angiogenic factor in veterinary practice.

Materials and Methods

The experiment was conducted at experimental sheds of the Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore. A total of 225, day old broiler chicks collected from the "Pakistan Hatchery" were vaccinated according to the vaccination schedule given in Table 1. The dipyrone (Metamizole sodium) injection 250 mg/ml was obtained from Orient labs. Pvt. Limited.

Table 1. Vaccination schedule.

Age	Vaccine	Route of Vaccination
6th day	Newcastle disease	Eye drop
15th day	Gumboro (I.B.D)	Drinking water
21st day	Newcastle disease	Drinking water
25th day	Gumboro (I.B.D)	Drinking water

Experimental design

On 28th day 75 birds were randomly divided into 2 groups comprising; Group A with 50 and B with 25 birds. On 29th day metamizole I/M 50mg/kg body weight injected for twice a day upto four days individually to each bird of group A, for four consecutive days. Group B was kept as control. The remaining 75 birds were divided into two groups C with 75 and D with 25 birds. Each bird of group C was injected metamizole I/M 100 mg/kg body weight for twice a day upto four days and group D was kept as control group and no medication given to them. Findings are have been summarized Table-5. Feed and water was provided *ad libitum*. A daily basis record of physical examination, sign and symptoms and toxicity was maintained regularly.

Sample schedule and parameters determined

Three ml blood sample from birds of each group (A, B, C and D) was collected before the start of medication on 29th day. Then blood sample from the same birds were drawn from wing vein (vena cutanea ulnaris) on days 33, 37 and 41 after medication for determination of serum values of following parameters; Aspartate Transaminase (Thomas, 1998), Alanine Transaminase (Thomas, 1998), Uric acid, Alkaline phosphatase (ALP) (Bessey *et al*., 1946), Concentration of creatinine in serum (Tietz, 1986)

Clinical findings and statistical analysis

The clinical findings mortality and postmortem was recorded during experimental period. The collected data were analyzed statistically with one way analysis of variance (Steel et. Al., 1982). On days 41st and 47th postmortem were done. Three parameters including postmortem, liver, kidney biopsies and stainings were examined.

Results

After completion of study, results colleted are given below. Groups B and D were kept control, as no medication was given to them and their findings are summarized in Table-6.

As shown in table 3 (a) the mean values of uric acid of metamizole sodium or dipyron was 5.945620 mg/dl, 5.669880 mg/dl, 6.552200 mg/dl and 5.847080 mg/dl at before medication, 1st day after medication, 5th and 9th day after medication respectively. There was no significant difference in the mean values of uric acid of dipyron table-3 (b).

The mean values of creatinine in metamizole sodium group was 1.387720 mg/dl, 1.247820 mg/dl, 1.411000 mg/dl and 1.101740 mg/dl at before medication, 1st day after medication, 5th and 9th day after

medication respectively table-3 (a). There was no significant difference in the mean values of creatinine of metamizole sodium table-3 (b).

As shown in table 3 (a) the mean values of alanine transaminase of metamizole sodium group was 10.328800 µ/L, 6.962000 µ/L, 10.556020 µ/L and 14.332860 µ/L at before medication, 1st day after medication, 5th and 9th day after medication respectively table-3 (b). There was significant difference in the mean values, of alanine transaminase of metamizole sodium table-3 (b).

As shown in table 3 (a) the mean values of aspartate transaminase of metamizole sodium was 173.64524 µ/L, 225.46830 µ/L, 217.304000 µ/L and 218.45800 µ/L at before medication, 1st day after medication, 5th and 9th day after medication respectively. There was significant difference in the mean values of aspartate transaminase of metamizole sodium table-3 (b).

As shown in table 3 (a) the mean values of alkaline phosphatase of metamizole sodium was 28.618000 µ/L, 48.470000 µ/L, 51.012000 µ/L and 47.072000 µ/L at before medication, 1st day after medication, 5th and 9th day after medication. There was significant difference in the mean values of alkaline phosphatase of metamizole sodium table- 3(b).

Table-3 (a):Various biochemical parameters of Dipyrone group n=5 values given as mean± SEM

Uric Acid mg/dl	Creatinine mg/dl	ALT µ/L	AST µ/L	ALP µ/L	Time of sample collection
5.9456200 ±.363993	1.387720 ±.162545	10.328800 ±.953864	173.64524 ±11.240398	28.618000 ±1.484148	Before medication
5.669880 ±.146420	1.247820 ±.108414	6.962000 ±.712966	225.46830 ±12.117352	48.470000 ±.977369	1st day after medication
6.552200 ±.378473	1.411000 ± 9.941EE-02	10.556020 ±.709688	217.30400 ±13.145889	51.012000 ±.924751	5th day after medication
5.847080 ±.256815	1.101740 ±7.530E-02	14.332860 ±.984038	218.45800 ±13.156353	47.072000 ±.886304	9th day after medication

Table-3 (b): ANOVA of Dipyrone

		Sum of Squares	Df	Mean Square	F	Sig.
Uric acid	Between groups	2.201	3	.734	1.616	.225
	Within groups	7.263	16	.454		
	Total	9.463	19			
Creatinine	Between groups	.307	3	.102	1.523	.247
	Within groups	1.075	16	6.716E-02		
	Total	1.381	19			
ALT	Between groups	136.163	3	45.388	12.563	.000
	Within groups	57.803	16	3.613		
	Total	193.966	19			
AST	Between groups	8396.290	3	2798.763	3.617	.036
	Within groups	12381.615	16	773.851		
	Total	20777.906	19			
ALP	Between groups	1575.104	3	525.035	87.531	.000
	Within groups	95.973	16	5.998		
	Total	1671.077	19			

Various biochemical parameters in control group

No medication was given to control group

As shown in table 5 (a) the mean values of uric acid of normal or control bird was 5.031800 mg/dl, 4.776720 mg/dl, 5.479140 mg/dl and 4.874880 mg/dl at 1st, 5th and 9th day. There was no significant difference in the mean values of uric acid of control birds table-5 (b).

The mean values of creatinine in control birds was 1.052080 mg/dl, 0.972660 mg/dl, 1.134440 mg/dl and 1.066040 mg/dl at 1st, 5th and 9th day. There was no significant difference in the mean values of creatinine in control birds table-5 (b).

As shown in table 5 (a) the mean values of alanine transaminase of control birds was 10.149740 µ/L, 10.205000 µ/L, 10.269660 µ/L and 10.351780 µ/L at 1st, 5th and 9th day. There was no significant difference in the mean values of ALT in control birds table-5 (b).

As shown in table 5 (a) the mean values of aspartate transaminase of control birds was 193.55568 µ/L, 199.43520 µ/L, 158.98660 µ/L and 166.81000 µ/L. There was no significant difference in the mean values of aspartate transaminase of control birds table-5 (b).

Table-5 (a): Various biochemical parameters of normal group n=5 values given as mean± SEM

Uric Acid mg/dl	Creatinine mg/dl	ALT µ/L	AST µ/L	ALP µ/L	Time of sample collection
5.031800 ±.209874	1.052080 ±3.172E-02	10.149740 ±.687521	193.55568 ±2.584983	27.252000 ±1.187440	0 day
4.776720 ±.474997	0.972669 ±6.390E-02	10.205000 ±.376771	199.43520 ±18.580837	34.470000 ±3.520395	1st day
5.479140 ±.400607	1.134440 ±9.002E-02	10.269660 ±.401202	158.98660 ±16.616974	33.548000 ±3.204323	5th day
4.874880 ±.152974	1.066040 ±9.043E-02	10.351780 ±.507856	166.81000 ±10.338517	27.824400 ±1.302525	9th day

As shown in table 5 (a) the mean values of alkaline phosphatase of piroxicam was 27.252000 µ/L, 34.470000 µ/L, 33.548000 µ/L and 27.824400 µ/L. There was no significant difference in the mean values of alkaline phosphatase of control birds table- 5(b).

Table-5 (b): ANOVA of normal

		Sum of Squares	Df	Mean Square	F	Sig.
Uric acid	Between groups	1.447	3	.482	.851	.486
	Within groups	9.071	16	.567		
	Total	10.519	19			
Creatinine	Between groups	6.607E-02	3	2.202E-02	.824	.499
	Within groups	.427	16	2.671E-02		
	Total	.493	19			
ALT	Between groups	.113	3	3.780E-02	.029	.993
	Within groups	20.670	16	1.292		
	Total	20.784	19			
AST	Between groups	5883.275	3	1961.092	2.135	.136
	Within groups	14698.768	16	918.673		
	Total	20582.043	19			
ALP	Between groups	212.301	3	70.767	2.197	.128
	Within groups	515.349	16	32.209		
	Total	727.650	19			

Results of necropsy findings of experimental chicks

The birds were slaughtered at the end of experiment and different lesions in kidney, liver and muscles were recorded. Each bird of group B was injected I/M Dipyron 50 mg/kg body weight for twice a day upto four days. Each bird of group C was injectedI/M Dipyron 100 mg/kg body weight for twice a day upto four days. Findings are mentioned in table-6. Groups B and C were kept control no medication given to them and group 0 was kept control no medication given to them. Findings are mentioned in table-6.

Table-6: Result of necropsy findings of various drugs in broilers chicks

Drug	Dose	Postmortem lesions		
		Site of injection	Liver	Kidney
Dipyron	50 mg/kg n = 15	0/15	3/15	0/15
	100 mg/kg n = 15	4/15	6/15	0/15
Control	No medication n = 15	0/15	0/15	0/15
	No medication n = 15	0/15	0/15	0/15

Discussion

Postmortem findings revealed that there were no hepatic or renal abnormalities moreover no muscle necrosis was observed at the injection site at the dosage rate of 1 mg/kg while muscle necrosis was observed in few birds at the dosage rate of the 2 mg/kg and the liver and kidneys were normal at the stated dose. The biochemical analysis showed that there was no change in serum uric acid, creatinine, ALT and AST levels in the samples collected at different times during experiment but there was significant difference in the values of ALP in the samples collected before and after medication which may be due to biliary obstructions Embert H., 1986).

It has been reported that when diclofenac sodium treated carcasses were eaten by the vultures, heavy mortality was noted due to acute renal failure caused by severe visceral gout (Oaks *et al.*, 2004). Therefore a suitable replacement of diclofenac sodium was needed in veterinary practice. Taking this into account the present project was designed to study the toxicity of dipyrone to explore the non-hazardous drug to safe wild life.

Roelvink *et al.,* (1991) evaluated the analgesic and spasmolytic effects of dipyrone (Novalgin) I/V 2500 mg/100 kg body weight in a balloon-induced model of colic, using five ponies with caecal fistulae. Dipyrone had a good analgesic effect in only two of the ponies, starting after eight to 10 minutes and lasting for 50 minutes.

Zentella de Pina *et al.,* (1993) assayed dipyrone to search its capability in preventing the hepatic increase of triacylglycerols and thiobarbituric acid reactive substances, as an indication of lipid peroxidation, resulting from the acute intoxication of rats with ethanol. It was concluded that the dipyrone contributed to controlling hepatic lipid peroxidation, and hence the oxidative stress promoted by ethanol intoxication.

Espiridiao *et al.,* (1996) studied the morphological and biochemical action of dipyrone on the placenta of albino rats by means of karyometry of trophoblastic giant cells and by determinations of DNA, RNA and total protein contents. Karyometric results showed that the nuclear volumes of placental cells in rats treated with dipyrone during the first 3 periods were significantly greater than in control animals

Postmortem findings of the birds treated with dipyron revealed hepatic lesions as focal necrosis. The kidneys of these birds were found normal at the doses rate of 50 mg/kg body weight. The lesions were severe when dose was increased to 100 mg/kg body weight. Necrotic lesions in the muscles at the site of injection were observed in few birds. The serum uric acid and creatinine level remained unchanged during experiment indicating that there was no damage to the kidneys. The results of this study are in accordance with Farker *et al.,* (1995) and Espiridiao *et al.,* (1996) who reported that dipyron has no effect on renal clearance of creatinine.

There was significant difference in the values of serum level of ALT, AST and ALP, which indicated liver damage as observed in postmortem findings. The postmortem of the control group revealed no abnormalities particularly in liver, kidneys and muscles. Similarly there was no significant difference in the serum values of uric acid and creatinine ALT, AST and ALP in the samples collected at the different times during experiments in the group.

Thus it is concluded that dipyrone is comparatively non-toxic in avian species. No mortality was recorded in all groups. Based on the necropsy findings and biochemical analysis it was found that dipyrone was safest drug. More over, the toxicity based ranking of dipyrone shows comparative better position to control pyrexia and inflammation in poultry and veterinary practice. Keeping in view the environmental problem (vultures crises) it is also recommended to prescribe dipyrone that has good pharmacological effects in human medicine may be used instead of diclofenac sodium in clinical practice.

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